abcam

Product datasheet

Anti-FHL2 antibody [EPR17860-20] ab202584

KO VALIDATED Recombinant RabMAb

4 References 11 Images

Overview

Product name	Anti-FHL2 antibody [EPR17860-20]
Description	Rabbit monoclonal [EPR17860-20] to FHL2
Host species	Rabbit
Tested applications	**Suitable for:** WB, ICC/IF, IP
Species reactivity	**Reacts with:** Mouse, Rat, Human
Immunogen	Recombinant fragment. This information is proprietary to Abcam and/or its suppliers.
Positive control	WB: U-2 OS, K526, HeLa, SW480, PC-12 and HT1080 whole cell lysates; Human fetal heart lysate; Mouse and rat heart lysates. ICC/IF: A-673 and NIH/3T3 cells. IP: SW480 whole cell lysate. ICC/IF: U-2 OS cells.
General notes	This product is a recombinant monoclonal antibody, which offers several advantages including: - High batch-to-batch consistency and reproducibility - Improved sensitivity and specificity - Long-term security of supply - Animal-free production For more information **see here**. Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to **RabMAb® patents**.

Properties

Form	Liquid
Storage instructions	Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C long term. Avoid freeze / thaw cycle.
Storage buffer	pH: 7.2 Preservative: 0.01% Sodium azide Constituents: 59% PBS, 40% Glycerol, 0.05% BSA
Purity	Protein A purified
Clonality	Monoclonal
Clone number	EPR17860-20

Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab202584 in the following tested applications.

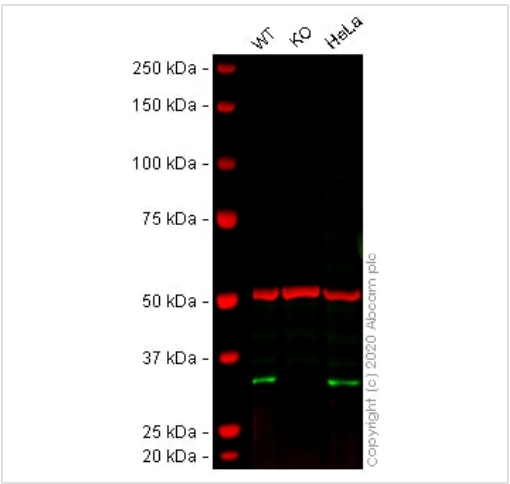
The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
WB		1/1000. Detects a band of approximately 32 kDa (predicted molecular weight: 32 kDa).
ICC/IF		1/400 - 1/200. ab202584 works both with PFA and methanol fixation. Fixation with PFA gives the strongest signal.
IP		1/30.

Target

Function	May function as a molecular transmitter linking various signaling pathways to transcriptional regulation. Negatively regulates the transcriptional repressor E4F1 and may function in cell growth.
Tissue specificity	Expressed in skeletal muscle and heart.
Sequence similarities	Contains 4 LIM zinc-binding domains.
Domain	The third LIM zinc-binding mediates interaction with E4F1.
Cellular localization	Cytoplasm. Nucleus.

Images



Western blot - Anti-FHL2 antibody [EPR17860-20] (ab202584)

All lanes : Anti-FHL2 antibody [EPR17860-20] (ab202584) at 1/1000 dilution

Lane 1 : Wild-type U-2 OS cell lysate
Lane 2 : FHL2 knockout U-2 OS cell lysate
Lane 3 : HeLa (Human epithelial cell line from cervix adenocarcinoma) whole cell lysate

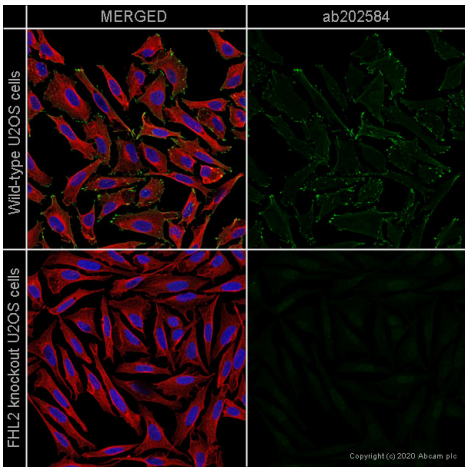
Lysates/proteins at 40 µg per lane.

Performed under reducing conditions.

Predicted band size: 32 kDa
Observed band size: 32 kDa

Lanes 1 - 3: Merged signal (red and green). Green - ab202584 observed at 32 kDa. Red - loading control **ab7291** (Mouse anti-Alpha Tubulin [DM1A] observed at 55kDa.

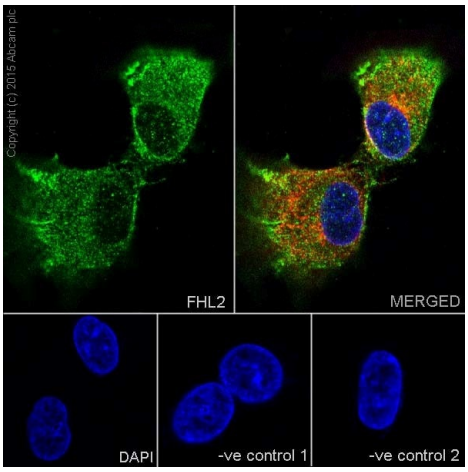
ab202584 was shown to react with FHL2 in wild-type U-2 OS cells in western blot with loss of signal observed in FHL2 knockout sample.Wild-type and FHL2 knockout U-2 OS cell lysates were subjected to SDS-PAGE. Membranes were blocked in 3% milk in TBS-T (0.1% Tween®) before incubation with ab202584 and **ab7291** (Mouse anti-Alpha Tubulin [DM1A] overnight at 4°C at a 1 in 1000 dilution and a 1 in 20000 dilution respectively. Blots were incubated with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preabsorbed (**ab216773**) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preabsorbed (**ab216776**) secondary antibodies at 1 in 20000 dilution for 1 hour at room temperature before imaging.



Immunocytochemistry/ Immunofluorescence - Anti-FHL2 antibody [EPR17860-20] (ab202584)

ab202584 staining FHL2 in wild-type U2OS cells (top panel) and FHL2 knockout U2OS cells (bottom panel). The cells were fixed with 4% PFA (10 min), permeabilized with 0.1% Triton X-100 for 5 minutes and then blocked with 1% BSA/10% normal goat serum/0.3M glycine in 0.1% PBS-Tween for 1h. The cells were then incubated with ab202584 at 1/200 dilution and **ab7291** (Tubulin) at 1/1000 dilution overnight at +4°C, followed by a further incubation at room temperature for 1h with a goat secondary antibody to rabbit IgG (Alexa Fluor® 488) (**ab150081**) at 2 µg/ml (shown in green) and a goat secondary antibody to mouse IgG (Alexa Fluor® 594) (**ab150120**) at 2 µg/ml (shown in pseudo color red). Nuclear DNA was labelled in blue with DAPI.

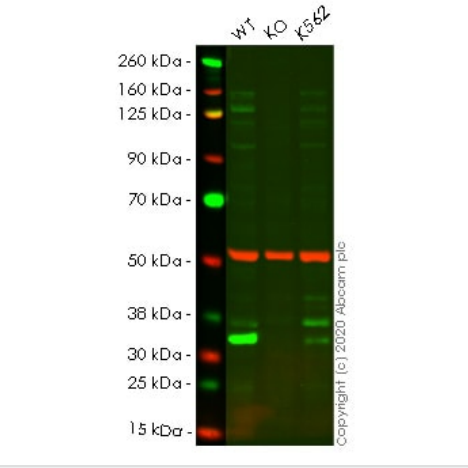
Image was taken with a confocal microscope (Leica-Microsystems, TCS SP8).



Immunocytochemistry/ Immunofluorescence - Anti-FHL2 antibody [EPR17860-20] (ab202584)

Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized A-673 (Human muscle Ewing's Sarcoma cell line) cells labeling FHL2 with ab202584 at 1/400 dilution, followed by Goat anti-rabbit IgG (Alexa Fluor® 488) (**ab150077**) secondary antibody at 1/500 dilution (green). Confocal image showing cytoplasmic and weakly nuclear staining on A-673 cell line. The nuclear counter stain is DAPI (blue). Tubulin is detected with **ab7291** (anti-Tubulin mouse mAb) at 1/1000 dilution and **ab150120** (AlexaFluor®594 Goat anti-Mouse secondary) at 1/500 dilution (red).

The negative controls are as follows:
-ve control 1: ab202584 at 1/400 dilution followed by **ab150120** (AlexaFluor®594 Goat anti-Mouse secondary) at 1/500 dilution.
-ve control 2: **ab7291** (anti-Tubulin mouse mAb) at 1/1000 dilution followed by **ab150077** (Alexa Fluor®488 Goat Anti-Rabbit IgG H&L) at 1/500 dilution.



Western blot - Anti-FHL2 antibody [EPR17860-20] (ab202584)

All lanes : Anti-FHL2 antibody [EPR17860-20] (ab202584) at 1/1000 dilution

Lane 1 : Wild-type HeLa lysate
Lane 2 : FHL2 knockout HeLa lysate
Lane 3 : K562 lysate

Lysates/proteins at 20 µg per lane.

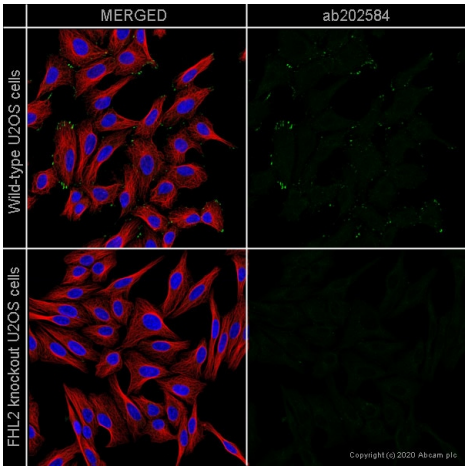
Performed under reducing conditions.

Predicted band size: 32 kDa

Lanes 1-3: Merged signal (red and green). Green - ab202584 observed at 32 kDa. Red - loading control **ab7291** observed at 50 kDa.

ab202584 Recombinant Anti-FHL2 antibody [EPR17860-20] was shown to specifically react with FHL2 in wild-type HeLa cells. Loss of signal was observed when knockout cell line **ab265475** (knockout cell lysate **ab257441**) was used. Wild-type and FHL2 knockout samples were subjected to SDS-PAGE. ab202584 and Anti-alpha Tubulin antibody [DM1A] - Loading Control?(**ab7291**) were incubated overnight at 4^°C at 1 in 1000 dilution and 1 in 20000 dilution respectively. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preadsorbed (**ab216773**) and

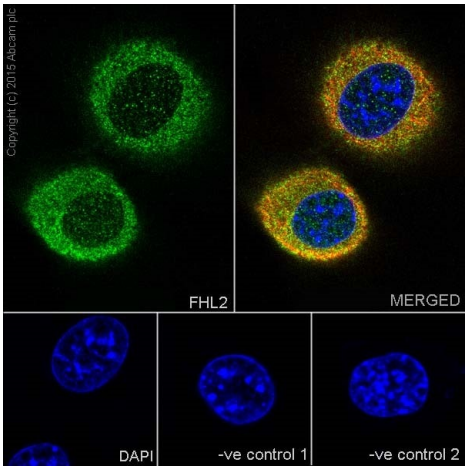
Goat anti-Mouse IgG H&L (IRDye® 680RD) preadsorbed (**ab216776**) secondary antibodies at 1 in 20000 dilution for 1 hour at room temperature before imaging.



Immunocytochemistry/ Immunofluorescence - Anti-FHL2 antibody [EPR17860-20] (ab202584)

ab202584 staining FHL2 in wild-type U2OS cells (top panel) and FHL2 knockout U2OS cells (bottom panel). The cells were fixed with 100% methanol (5 min), permeabilized with 0.1% Triton X-100 for 5 minutes and then blocked with 1% BSA/10% normal goat serum/0.3M glycine in 0.1% PBS-Tween for 1h. The cells were then incubated with ab202584 at 1/200 dilution and **ab7291** (Tubulin) at 1/1000 dilution overnight at +4°C, followed by a further incubation at room temperature for 1h with a goat secondary antibody to rabbit IgG (Alexa Fluor® 488) (**ab150081**) at 2 µg/ml (shown in green) and a goat secondary antibody to mouse IgG (Alexa Fluor® 594) (**ab150120**) at 2 µg/ml (shown in pseudo color red). Nuclear DNA was labelled in blue with DAPI.

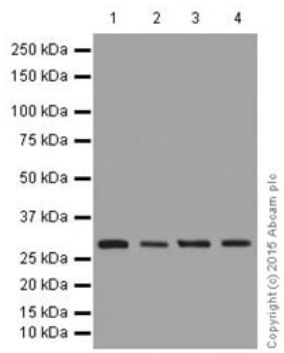
Image was taken with a confocal microscope (Leica-Microsystems, TCS SP8).



Immunocytochemistry/ Immunofluorescence - Anti-FHL2 antibody [EPR17860-20] (ab202584)

Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized NIH/3T3 (Mouse embyro fibroblast cells) cells labeling FHL2 with ab202584 at 1/400 dilution, followed by Goat anti-rabbit IgG (Alexa Fluor® 488) (**ab150077**) secondary antibody at 1/500 dilution (green). Confocal image showing cytoplasmic and weakly nuclear staining on NIH/3T3 cell line. The nuclear counter stain is DAPI (blue). Tubulin is detected with **ab7291** (anti-Tubulin mouse mAb) at 1/1000 dilution and **ab150120** (AlexaFluor®594 Goat anti-Mouse secondary) at 1/500 dilution (red).

The negative controls are as follows:
-ve control 1: ab202584 at 1/400 dilution followed by **ab150120** (AlexaFluor®594 Goat anti-Mouse secondary) at 1/500 dilution.
-ve control 2: **ab7291** (anti-Tubulin mouse mAb) at 1/1000 dilution followed by **ab150077** (Alexa Fluor®488 Goat Anti-Rabbit IgG H&L) at 1/500 dilution.



Western blot - Anti-FHL2 antibody [EPR17860-20] (ab202584)

All lanes : Anti-FHL2 antibody [EPR17860-20] (ab202584) at 1/1000 dilution

Lane 1 : SW480 (Human colorectal adenocarcinoma cell line) whole cell lysate
Lane 2 : PC-12 (Rat adrenal gland pheochromocytoma) whole cell lysate
Lane 3 : HT1080 (Human fibrosarcoma cells) whole cell lysate
Lane 4 : HeLa (Human epithelial cells from cervix adenocarcinoma) whole cell lysate

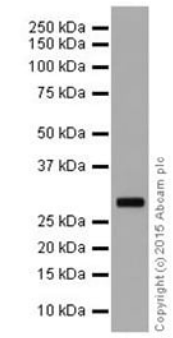
Lysates/proteins at 10 µg per lane.

Secondary
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/1000 dilution (Goat Anti-Rabbit IgG, (H+L), Peroxidase conjugated)

Predicted band size: 32 kDa
Observed band size: 32 kDa

Exposure time: 30 seconds

Blocking/Dilution buffer: 5% NFDM/TBST.



Western blot - Anti-FHL2 antibody [EPR17860-20] (ab202584)

Anti-FHL2 antibody [EPR17860-20] (ab202584) at 1/10000 dilution + Human fetal heart lysate at 10 µg

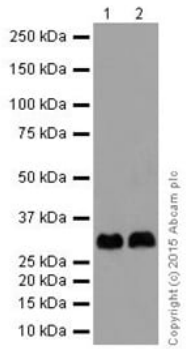
Secondary

Anti-Rabbit IgG (HRP), specific to the non-reduced form of IgG at 1/1000 dilution

Predicted band size: 32 kDa
Observed band size: 32 kDa

Exposure time: 15 seconds

Blocking/Dilution buffer: 5% NFDM/TBST.



Western blot - Anti-FHL2 antibody [EPR17860-20] (ab202584)

All lanes : Anti-FHL2 antibody [EPR17860-20] (ab202584) at 1/2000 dilution

Lane 1 : Mouse heart lysate
Lane 2 : Rat heart lysate

Lysates/proteins at 10 µg per lane.

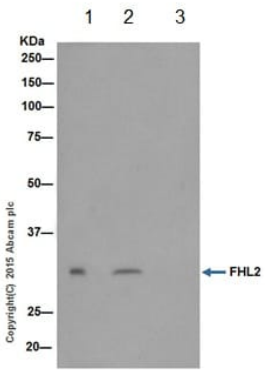
Secondary

All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/1000 dilution (Goat Anti-Rabbit IgG, (H+L), Peroxidase conjugated)

Predicted band size: 32 kDa
Observed band size: 32 kDa

Exposure time: 10 seconds

Blocking/Dilution buffer: 5% NFDM/TBST.



Immunoprecipitation - Anti-FHL2 antibody [EPR17860-20] (ab202584)

FHL2 was immunoprecipitated from 1mg of SW480 (Human colorectal adenocarcinoma cell line) whole cell lysate with ab202584 at 1/30 dilution. Western blot was performed from the immunoprecipitate using ab202584 at 1/1000 dilution. Anti-Rabbit IgG (HRP), specific to the non-reduced form of IgG, was used as secondary antibody at 1/1500 dilution.

Lane 1: SW480 whole cell lysate 10 µg (Input). Lane 2: ab202584 IP in SW480 whole cell lysate. Lane 3: Rabbit monoclonal IgG (**ab172730**) instead of ab202584 in SW480 whole cell lysate.

Blocking and dilution buffer and concentration: 5% NFDM/TBST.

Exposure time: 10 seconds.

Anti-FHL2 antibody [EPR17860-20] (ab202584)

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